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(54) Title: VITAMIN DIRECTED DUAL TARGETING THERAPY

(57) Abstract: The invention relates to vitamin-mediated targeting for the delivery of agents and active substances in the therapy of disease. Combined targeting using vitamins essential for cancer growth are used in complexes of the invention for the amplified delivery of cytotoxic drugs to tumors and cancer cells, with a concomitant reduction in toxicity to the subject being treated.

VITAMIN DIRECTED DUAL TARGETING THERAPY

Technical Field

The invention relates to vitamin-mediated targeting for the delivery of agents and active substances in the therapy of disease. More particularly the invention relates to combined targeting using vitamins essential for cancer growth in vitamin complexes used to deliver cytotoxic drugs to tumors and cancer cells. The invention also relates to processes for preparing the complexes, pharmaceutical compositions containing same, methods of treatment involving the complexes and uses of the complexes in the manufacture of medicaments.

Background Art

Chemotherapy has been, for many decades, one of the major approaches to the control and cure of malignant neoplasms (cancers). In conventional cancer chemotherapy it is often necessary to increase the quantity of cytotoxic drugs administered in an exponential fashion in order to obtain a linear increase in the death of cancer cells. This in turn leads to an undesirable increase in non-specific cytotoxicity of bystander, healthy cells. Hence it is often necessary to repeatedly deliver smaller doses of cytotoxin, which inevitably leads to the survival of a small fraction of drug-resistant cells. This in turn has necessitated the development of more specific and stronger cytotoxic drugs, however their nonselective action on cells other than cancerous cells still remains a major problem.

In an attempt to increase the dose of cytotoxic agent delivered to the tumor cell, specific targeting agents such as monoclonal antibodies to "tumor-specific antigens" have been employed. While this therapy has proven to produce more specific targeting, in many instances it has not been possible to deliver enough cytotoxic agent coupled to the antibody for effective tumoricidal activity.

Recently focus has switched to the use of molecules essential for growth to be used as targeting agents. In particular, research has concentrated on the use of vitamins and in particular folic acid and vitamin B_{12} (cobalamin, Cbl, VB_{12}) for tumour specific targeting.

Folic acid enters cells either through a carrier protein, termed the reduced folate carrier, or via receptor-mediated endocytosis facilitated by folate receptors (FR). Folate receptors are significantly over-expressed on a large proportion of human cancer cells including ovarian, breast, lung, endometrial, renal, colon, and cancers of myeloid hematopoietic cells. There are two folate receptors, FR-α, and FR-β. In general FR-α is upregulated in malignant tissues of epithelial origin such as ovarian carcinoma, while FR-β is overexpressed in malignant tissues of non-epithelial origin. While the FR have been detected in normal tissues involved in the retention and uptake of the vitamin, these tissues are in protected sites and generally not accessible following blood-borne delivery of folate conjugates. Thus there is expression in the choroid plexus, the intestinal brush border apical membrane surface and the proximal tubules of the kidney. In the latter case the receptor probably functions to scavenge excreted folate, and as such would not be accessible to large molecular weight folate complexes. Folate-mediated tumor targeting has been exploited to date for delivery of the following molecules and molecular complexes (i) protein toxins, (ii) low-molecular-weight chemotherapeutic agents, (iii) radio-imaging agents (iv) MRI contrast agents, (v) radiotherapeutic agents, (vi) liposomes with entrapped drugs, (vii) genes, (viii) antisense oligonucleotides, (ix) ribozymes, and (x) immunotherapeutic agents.

Cellular uptake of vitamin B_{12} (cobalamin, Cbl, VB_{12}) is mediated primarily by the plasma protein transcolbamin II (TCII). Following binding of Cbl to TCII the resultant TCII-Cbl complex binds with high affinity to receptors on the surface of cells and is internalized by the cell via a process called receptor-mediated endocytosis (RME). Once inside the cell the Cbl is enzymatically modified to form two coenzymes, which are in turn for two essential metabolic pathways. One involves the methylation of homocysteine in the *de novo* synthesis of methionine, and is catalyzed by methionine synthetase. The other

pathway involves the rearrangement of methyl malonyl CoA to succinyl CoA, and is catalyzed by methyl malonyl CoA mutase. It has recently been shown that the *in vitro* proliferation of human and murine leukemia cells is dependent upon both TCII and Cbl (see McLean, G. R., Quadros, E. B., Rothenberg, S. P., Morgan, A. C., Schrader, J. W., and Ziltener, H. J., Antibodies to transcobalamin II block *in vitro* proliferation of leukemic cells, *Blood,* 1997, *89,* 235-242). Several workers have now concentrated on utilizing Cbl conjugates for both radio-imaging and for targeted cancer chemotherapy (see Smeltzer, C. C., Pinson, P. R., Munger, J. M., West, F. G., and Grissom, C. B., Cytotoxicities of two new cobalamin bioconjugates, *Proceedings Ninth International Symposium on Recent Advances in Drug Delivery Systems,* 1999, pp 232-3; Canon, M.J., Munger, J. M., West, F. G., and Grissom, C. B., Synthesis and uptake of radiolabeled cobalamin bioconjugate, *Proceedings Ninth International Symposium on Recent Advances in Drug Delivery Systems,* 1999, pp 230-1; and Pinson, P. R., Munger, J. M., West, F. G., and Grissom, C. B., Synthesis of two doxorubicin-cobalamin bioconjugates, *Proceedings Ninth International Symposium on Recent Advances in Drug Delivery Systems,* 1999, pp 228-9).

However, there is one major limitation to the use of vitamins as molecules to target to tumor cells, and that is the targeting of each molecule is not entirely specific for tumour cells. For example the majority of folate-drug complexes are very small and as such are excreted in the kidneys and re-absorbed in the proximal tubules, thus leading to undesirable accumulation of folate-drug complexes in the kidney. Similarly for VB_{12}-drug complexes there is undesirable accumulation in the liver.

Limitations of the vitamin complexes are partly addressed by the production of large molecular weight polymer complexes between the vitamin and the agent or active substance to be delivered. Thus, amplification of drug delivery can occur by linkage of a drug/ pharmaceutical to a bio-compatible polymer backbone to which a number of vitamin molecules are linked, either subsequently, previously or concurrently. Preferably the linkage to the polymer, or the polymer to which the pharmaceutical is linked, should be biodegradable. This strategy is the subject of a folate-polymer complex patent

application (PCT/AU00/00406, filed 4 May 2000) and a VB_{12}-polymer complex patent (Russell-Jones *et al.*, 1995 USP 5,449,720) both specifications of which are incorporated herein in their entirety by reference. Alternatively the drug could be included within a vitamin-coated nanoparticle, which strategy is the subject of a folate-nanoparticle patent application (PCT/AU00/00405, filed 4 May 2000) and a VB_{12}-nanoparticle patent (Russell-Jones *et al.*, 1997 EP 0,531,497 B1) both specifications of which are incorporated herein in their entirety by reference. Nonetheless there is still a need for better and more effective drug targeting to sites such as tumor tissues and cancer cells.

Thus it is an object of the present invention to overcome, or at least alleviate one or more of the above mentioned disadvantages of the prior art.

Summary of the Invention

Surprisingly it has been found by the present inventors that amplification of agent or active substance delivery can be enhanced by the combined use of two or more different vitamin complexes. By administering different vitamin-complexes it is possible to target and deliver greater amounts of agents and active substances such as drugs and pharmaceuticals to tumor tissues and cancer cells, whilst at the same time minimising the unwanted accumulation of these agents at other sites or in organs such as the kidneys (in particular folate complexes) and liver (in particular VB_{12} complexes). This greatly reduces the toxicity of the drug or pharmaceutical being delivered.

Thus according to a first aspect of the present invention there is provided a pharmaceutical composition comprising a first vitamin complex and a second vitamin complex wherein the vitamin of the first complex is different to the vitamin of the second complex and wherein both vitamins are complexed to the same agent or active substance.

According to a second aspect of the present invention there is provided a pharmaceutical composition comprising a first vitamin complex and a second vitamin complex wherein

the vitamin of the first complex is different to the vitamin of the second complex and wherein both vitamins are each complexed to different agents or active substances.

According to a third aspect of the present invention there is provided a method for the manufacture of a pharmaceutical composition of the present invention which method comprises admixing a first vitamin complex, one or more further vitamin complexes and a pharmaceutically acceptable carrier, excipient, diluent and/or adjuvant.

According to a fourth aspect of the present invention there is a pharmaceutical composition prepared by a method of the third aspect.

According to a fifth aspect of the present invention there is provided a method for the treatment, prophylaxis or amelioration of disease, preferably cancer, which comprises administering to a subject a therapeutically effective amount of a first vitamin complex and a second vitamin complex wherein the vitamin of the first complex is different to the vitamin of the second complex and wherein both vitamins are complexed to the same agent or active substance.

According to a sixth aspect of the present invention there is provided a method for the treatment, prophylaxis or amelioration of disease, preferably cancer, which comprises administering to a subject a therapeutically effective amount of a first vitamin complex and a second vitamin complex wherein the vitamin of the first complex is different to the vitamin of the second complex and wherein both vitamins are each complexed to different agents or active substances.

According to a seventh aspect of the present invention there is provided use of a first vitamin complex and a second vitamin complex wherein the vitamin of the first complex is different to the vitamin of the second complex and wherein both vitamins are complexed to the same agent or active substance in the preparation of a medicament for the treatment, prophylaxis or amelioration of disease, preferably cancer.

According to a eighth aspect of the present invention there is provided use of a first vitamin complex and a second vitamin complex wherein the vitamin of the first complex is different to the vitamin of the second complex and wherein both vitamins are each complexed to different agents or active substances in the manufacture of a medicament for the treatment, prophylaxis or amelioration of disease, preferably cancer.

According to a ninth aspect of the present invention there is provided a kit for the delivery of one or more agents or active substances to a subject, said kit comprising a first vitamin complex and a second vitamin complex wherein the vitamin of the first complex is different to the vitamin of the second complex and wherein both vitamins are complexed to the same agent or active substance.

According to a tenth aspect of the present invention there is provided a kit for the delivery of agents or active substances to a subject, said kit comprising a first vitamin complex and a second vitamin complex wherein the vitamin of the first complex is different to the vitamin of the second complex and wherein both vitamins are each complexed to different agents or active substances.

The pharmaceutical compositions of the present invention may further comprise a pharmaceutically acceptable carrier, excipient, diluent and/or adjuvant and optionally one or more further vitamin complexes either the same as or different from either of the first or second vitamin complexes of the composition.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

Brief Description of the Figure

The present invention will now be described by way of example only and with reference to the figure wherein:

Figure 1 represents the targeting of a tumor with a pharmaceutical composition of the present invention comprising a Cbl-targeting complex and a folate-targeting complex.

In Figure 1, a subject is depicted in which the star (1) represents a tumor, the crescent (2) represents the liver and the ovals (3) represent the kidneys. Figure 1A shows the targeting of a tumor with a Cbl complex and the unwanted accumulation of the Cbl complex in the liver. Figure 1B shows the targeting of a tumor with a folate complex and the unwanted accumulation of the folate complex in the kidneys. Figure 1C shows the dual targeting of a tumor with a folate complex/Cbl complex composition of the present invention. It can be seen in Figure 1C that there is an amplification of agent or drug/pharmaceutical delivery to the tumor with respect to the delivery to either the kidneys or liver, which in turn leads to a relative reduction in the toxicity of the cytotoxic complexes to the subject being treated.

Detailed Description of the Invention

An advantage provided by the present invention is the ability to amplify agent or active substance delivery to a subject by the use of different vitamin complexes. The vitamin complexes preferentially target tumor tissues and cancer cells for it is in these tissues and cells that there often is an upregulation of vitamin receptors. Vitamins suitable for making vitamin complexes include, amongst others, folic acid, VB_{12}, riboflavin and biotin, more preferably folic acid and VB_{12}. It will be understood that derivatives and analogues of vitamins are also within the scope of the present invention. Analogues contemplated herein include, but are not limited to, modification to the ring structure, functional groups or side chains of the vitamin molecule including the additional removal of protecting groups and salts and complexes thereof derived from any source such as

being chemically synthesized or identified by screening process such as natural product screening provided that the analogue possesses some binding activity for the vitamin receptor. It will be understood by those skilled in the art that upregulated receptors other than just the vitamin receptors on tumor or cancer cells can be targeted by the complexes of the present invention.

Administration of the pharmaceutical compositions of the present invention is either by the simultaneous or sequential administration of vitamin complexes, preferably a folate complex and a VB_{12} complex. The vitamin complexes deliver agents or active substances, in particular hormones, drugs, prodrugs, enzymes, proteins, peptides, toxins, immunogens or DNA or RNA analogues to subjects.

Suitable toxins for use in the invention include, but are not limited to, ricin, abrin, diphtheria toxin, modecin, tetanus toxin, mycotoxins, mellitin, α-amanitin, pokeweed antiviral protein, ribosome inhibiting proteins, especially those of wheat, barley, corn, rye, gelonin and maytansinoid.

Suitable cytotoxic agents for use in the invention include, but are not limited to, alkylating agents such as chlorambucil, cyclophosphamide, melphalan, cyclopropane; anthracycline antitumor antibiotics such as doxorubicin, daunomycin, adriamycin, mitomycin C, 2-(hydroxymethyl)anthraquinone; antimetabolites such as methotrexate, dichloromethatrexate: cisplatin, carboplatin, and metallopeptides containing platinum, copper, vanadium, iron, cobalt, gold, cadmium, zinc and nickel. Other agents include DON, thymidine, pentamethylmelamin, dianhydrogalactitol, 5-Methyl-THF, anguidine, maytansine, neocarzinostatin, chlorozotocin, AZQ, 2'deoxycoformycin, PALA, AD-32, *m*-AMSA and misonidazole.

In one embodiment of the invention different vitamins are conjugated to the same type of active substance. Administration of the vitamin complexes so produced results in the complexes targeting cancer cells which are upregulated for those vitamins whilst at the same time unwanted accumulation of the vitamin complexes is spread over different

organs and tissues which also happen to be targeted by the particular vitamins. This results in the amplification of delivery of active substances, such as cytotoxins, to the target cells thereby greatly reducing the toxicity of the cytotoxic conjugates to other parts of the body.

In another embodiment different vitamins are conjugated to different active substances. This also results in the amplification of, for example, cytotoxic agents to target cells in combination drug therapy.

In an important embodiment of the present invention it is possible to administer a first vitamin conjugate with a relatively non-toxic prodrug of a cytotoxic agent together with or sequentially to a second vitamin conjugate with a relatively non-toxic enzyme. The first vitamin conjugate may for example use folic acid as the targeting vitamin. The folate moiety targets the tumor tissue to be treated and also happens to target the kidneys, delivering the prodrug rather than the cytotoxic agent itself. Sequential or simultaneous administration of the second vitamin conjugate using for example a VB_{12}-enzyme complex will target the tumor and the liver, as a result of the VB_{12} targeting. The enzyme is adapted to transform the prodrug into a cytotoxic agent which directly acts on the targeted tumor tissue. The toxicities of the administered vitamin complexes are greatly reduced because substantially less cytotoxic drug will be synthesized in other tissues and organs in the treated subject. This is a result of some accumulation in the kidneys of relatively non-toxic folate-prodrug complex but little or no delivery of the VB_{12}-enzyme complex, whilst there is some accumulation of the relatively non-toxic VB_{12}-enzyme in the liver but little or no delivery of the folate-prodrug complex.

Most preferably the subject firstly receives a dose of an antibody-enzyme conjugate, after which the patient then receives a dose of a prodrug conjugate. A cytotoxic agent is then synthesised or released from the prodrug conjugate at the targeted tumor site by the action of the enzyme.

In a further embodiment of the present invention, the agents or active substances may be delivered by existing encapsulation methods or polymer-drug complexes. Suitable encapsulation methods utilising polymers in the preparation of biodegradable nanoparticles and microspheres, which has the targeting molecule attached to it, are described in the above mentioned folate-nanoparticle patent application (PCT/AU00/00405, filed 4 May 2000) and the VB_{12}-nanoparticle patent (Russell-Jones *et al.*, 1997 EP 0,531,497 B1) and are described below as follows:-

Polymers suitable for the formation of nanoparticles by solvent evaporation (in liquid drying) include, amongst others, poly-lactic acid, poly-(Lactide/co-glycolide), poly-hydroxybutyrate, poly-hydroxyvalerate, poly-(hydroxybutyrate/valerate), ethyl cellulose, dextran, polysaccharides, polyalkylcyanoacrylate, poly-methyl-methacrylate, poly(e-caprolactone) and various combinations and co-polymers of the above.

Polymers suitable for the formation of microspheres by interfacial precipitation/polymerization include, amongst others, EUDRAGIT™; Poly(N^a,N^e-L-lysinediylterephthaloyl); polymers formed by the reaction of Lysine hydrochloride and p-phthaloyl dichloride; by the reaction of acryloylated maltodextrin or acryloylated hydroxyethyl starch with ammonium peroxodisulfate and N,N,N',N'-tetramethylethylenediamine. Microspheres can also be formed by the polymerization of various diamines such as ethylene diamine, phenylenediamine, toluene diamine, hexamethylene diamine, or diols such as ethylene diol, bisphenol, resorcinol, catechol, pentanediol, hexanediol, dodecanediol, 1,4-butanediol, with diacid chlorides such as sebacoylchloride and adipoyl chloride, or diisocynates such as hexamethylene diisocyanate using the methods fully described in EP 85870002.4.

Polymers suitable for the formation of microspheres by polymer phase separation include co-poly(vinyl chloride:vinyl alcohol:vinyl acetate), cellulosic polymers, polyvinyl acetate, polyvinyl alcohol, polyvinylchloride, natural and synthetic rubbers, polyacrylates, polystyrene and the like. Methods to synthesize such microspheres are fully described in USP 4,166,800.

Polymers suitable for the formation of microspheres by complex coacervation include, amongst others, mixtures of polyanions, such as gum arabic, alginate, carboxymethyl cellulose, carboxymethyl starch, polystyrene sulfonic acid, polyvinyl sulfonic acid, poly-d-glucuronic acid, poly-pyruvic acid, carrageenan, heparin sulphate, polyphosphate with polycations, such as polylysine, gelatin.

Polymers suitable for the formation of microspheres by Polymer/Polymer incompatibility include, amongst others, ethyl cellulose, ethylene vinyl acetate polymer, poly(lactide), or poly(vinylidene chloride) mixed with polymers such as polyethylene, silicone, polyisobutylene or polybutadiene.

Other materials suitable for formation of microspheres include, starch, cross-linked albumen, polyacrylamide, cross-linked gelatin and others known to those skilled in the art of microsphere preparation.

Suitable polymer-drug complexes utilise targeting molecules to deliver agents or active substances as described in the above mentioned folate-polymer complex patent application (PCT/AU00/00406, filed 4 May 2000) and the VB_{12}-polymer complex patent (Russell-Jones *et al.*, 1995 USP 5,449,720) and are described below as follows:-

Polymers useful according to the invention include potentially biodegradable polymers such as dextran and its derivatives, amino acid polymers such poly-lysine, poly-glutamic acid. Non-biodegradable polymers include poly[N-(2-hydroxypropyl)-methacrylamide], to which is attached biodegradable side chains such as those containing ester linkages, or amino acid sequences cleavable within lysosomal vacuoles i.e. Gly-Phe-Leu-Gly (see Rihova, B. and Kopecek J., Biological properties of targetable poly[N-(2-hydroxypropyl)-methacrylamide]-antibody complexes, *J. Control Rel.*, 1985, 2:289-310). Other amino acid spacers cleavable by intracellular proteases include Gly-Phe-Ala; Gly-Phe-Ala-Gly; Gly-Phe-Tyr-Ala; and Gly-Phe-Tyr-Ala-Ala (see Rejmanova, P., Obereigner, B., and Kopecek, J., *Makromol. Chem.*, 1981, 182:1899-1915).

Furthermore the vitamin targeting agents may be linked to polymers to which are linked various enzymes, drugs, and cytotoxic agents for the control of tumour growth. These targeting agent-polymer-drug conjugates are suitable for parenteral delivery to tumors as they can utilise the aforementioned targeting agent (TA) receptor system for uptake binding and uptake, and have the added advantage of increasing the amount of pharmaceutical agent which can be delivered via the TA uptake mechanism. A further advantage of using polymers in combination with folic acid, or an analogue thereof, as a TA, is their ability to avoid or at least reduce targeting to the kidneys by virtue of their size.

In one embodiment of the invention the linkage joining the pharmaceutical, or the TA to the polymer is a disulfide bond. In a further embodiment of the invention the linkage joining the pharmaceutical, or the TA to the polymer is an ester linkage. In yet another embodiment of the invention the linkage joining the pharmaceutical or the TA to the polymer is a γ-glutamyl-ε-lysine bond. In yet another embodiment of the invention the linkage joining the pharmaceutical or the TA to the polymer is a diazo-linkage.

In a preferred embodiment vitamin polymer complexes have the general formula:

$$(TA\text{-}Q)_n\text{-}P\text{-}(Q'\text{-}A)_m$$

wherein, TA, is either vitamin B_{12} or an analogue thereof which will bind via TCII to surface receptors on tumor cells, or where TA is folate or an analogue thereof which will bind via folate receptors to surface receptors on tumor cells, and where

n, the molar substitution ration of TA in the complex, is in the range from 1.0 to 50.0, and

P is a pharmaceutically acceptable polymer, and

A is a pharmaceutically active substance, and

m, the molar substitution ratio of A in the complex, is in the range from 1.0 to 1000, and

Q and Q' are independently a covalent bond, or a spacer compound linking folate, P and A by covalent bonds.

The polymer, P, of the present invention can be any pharmaceutically acceptable polymer. The polymer is able to attach to at least one carrier molecule and to at least one, but preferably a multiplicity of active substance molecules.

Suitable polymers for substitution with folate and modification according to the invention, include poly[N-(2-hydroxypropyl)-methacrylamide], dextran, chondroitan sulfate, water soluble polyurethanes formed by covalent linkage of PEG with lysine, poly(glutamic acid), poly(hydroxypropyl glutamine) and branched chain polypeptides formed by the dual modification of the α- and ε-amino groups of lysine during the peptide synthesis. Such polymers may have multiple amino-termini, to which can be conjugated a plurality of the pharmaceutical or drug to be delivered. The polymers can also be formed with multiple cystines, to provide free thiols, or multiple glutamates or aspartates, to provide free carboxyls for conjugation using suitable carbodiimides. Similarly the polymer can contain multiple histidines or tyrosines for conjugation.

Folate is most easily covalently attached to a ligand, or the polymer, via either its α or γ carboxylic acid moiety. It has been shown, however, that the α-carboxyl derivatives have low avidity for the folate receptor, whereas the γ-carboxy derivatives have similar affinity to native folate.

The spacer compounds Q and Q' are optional. When they are absent the carrier (folate or a suitable derivative of vitamin B_{12}) and/or the active substance A are linked to polymer P by a direct covalent bond. They are introduced either to improve the receptor affinity of the carrier complex or to overcome problems in the coupling of the carrier, and/or the active substance A arising from unfavourable steric interactions between the carrier and A with the polymer P, or to increase the bioactivity of A in the complex. The spacer compounds may also act as linking agents, being bi-functional compounds with selected functional groups on each end to react with suitable functional groups located on the polymer, and also on the vitamin carrier molecule and/or on the pharmaceutically active substances.

Polymers to which are conjugated various cytotoxic drugs have been previously described. These polymers have been targeted to cancer cells using for example specific antibodies or sugar moieties. Once the drug-polymer has reached its target tissue the complex is endocytosed by the target cell and the pendant drug is released by the action of lysosomal enzymes, or by cleavage of a disulfide-linked drug by intracellular glutathione.

It will be evident to a person skilled in the art that different variations of vitamin conjugate pairs can be used provided that different vitamins target and deliver, either directly or indirectly, agents or active substances to the same site to be treated. Preferred vitamin complex pairs include, amongst others, the following:

a) Cbl-enzyme + Folate-substrate-cytotoxin
b) Folate-enzyme + Cbl-substrate-cytotoxin
c) Cbl-enzyme + Folate-Polymer-substrate-cytotoxin
d) Folate-enzyme + Cbl-Polymer-substrate-cytotoxin
e) Cbl-enzyme + Folate-NP-substrate-cytotoxin
f) Folate-enzyme + Cbl-NP-substrate-cytotoxin
g) Cbl-NP-Enzyme + Folate-NP-substrate-cytotoxin

Particularly preferred enzyme prodrug combinations suitable for the invention include the following peptidase substrate pairs:-

- Carboxypeptidase G2 in combination with prodrugs of nitrogen mustard alkylating agents (Bagshawe, K. C. 1987 *Br J. Cancer, 56, 531*; Bagshawe, K. C., Springer, C. M., Searle, F., Antoniw, P., Sharma, S. K., Melton, R. G., Sherwood, R. F. 1988 *Br. J. cancer, 58, 700*; Searle, F., Bier, C., Buckley, R. G., Newman, S., Pedley, R. B., Bagshawe, K. D., Melton, R. G., Alwan, S. M., Sherwood, R. F. 1986, *Br. J. Cancer, 53, 377*; Springer, C. J., Antoniw, P., Bagshawe, K. C., Searle, F., Bisset, G. M. F., Jarman, M. 1990 *J. Med. Chem., 33, 677*).

- Carboxypeptidase A or B and mono- or dipeptide prodrugs of methotrexate, such as alanyl-MTX.
- Penicillin V/G amidase in combination with *p*-hydroxyphenoxy-derivatives of doxorubicin and melphalan.
- Penicillin G amidase in combination with phenylacetamides of doxorubicin, melphalan and palytoxin.
- β-Lactamases and derivatives of cephalosporins and methotrexate, or cephalosporins and 5-fluorouracil, or alternatively cephalosporins and analogues of vinblastine. Others include prodrugs formed with cephalosporins and doxorubicin, carboplatin analogues and taxol.
- Alkaline phosphatase and phosphate prodrugs of etoposide (etoposide phosphate), mitomycin C (mitomycin phosphate), phenol mustard, nitrogen mustard, and doxorubicin (doxorubicin phosphate) (Senter, P.D. 1990 Activation of prodrugs by antibody-enzyme conjugates: a new approach to cancer therapy. *FASEB J., 4, 188-193).*

Further preferred enzyme prodrug combinations suitable for the invention include the following:-

- β-Glucuronidase and glucuronic acid derivatives of nitrogen mustard analogues, phenol mustard analogues,
- α-Galactosidase and glycosidic derivatives of daunorubicin.
- Cytosine deaminase in combination with 5-fluorocytosine.
- Nitroreductase and 5-(aziridin-1-yl)-2,4-dinitrobenzamide, actinomycin, or mitomycin.
- Arylsulfatase and sulfate derivatives of etoposide or phenol mustard
- β- Galactosidase and glycosidic derivatives of daunorubicin

The vitamin complexes used in the present invention may also be formed from more than one active substance linked to a polymer, which is linked to at least one targeting agent (vitamin). The ability of the targeting agent to undergo the binding reactions necessary for uptake and transport of the active substance in a vertebrate host and the activity of the active substance are substantially maintained, following conjugation or following biological release of the active substance from the polymer. Suitable methods for the manufacture of these vitamin complexes comprises one or more of the following steps:

a) reacting the active substance with the polymer to form said complex;

b) chemically modifying the active substance to provide at least one functional group capable of forming a chemical linkage, and reacting the active substance and polymer to form said complex;

c) chemically modifying the targeting agent to provide at least one functional group capable of forming a chemical linkage and reacting the targeting agent and polymer to form said complex;

d) chemically modifying the active substance and the polymer to provide functional groups capable of forming a chemical linkage, and reacting the active substance and polymer to form said complex;

e) reacting the active substance with at least one cross-linking agent and reacting the active substance of polymer to form said complex;

f) reacting the targeting agent with at least one cross-linking agent and reacting the polymer and carrier to form said complex;

g) reacting the active substance and polymer with at least one cross-linking agent and reacting the active substance and polymer to form said complex;

h) reacting the active substance directly with a polymeric support to form an intermediate containing one or more molecules of the active substance linked to the polymer, and subsequently coupling the polymer-active substance intermediate to one or more targeting agents; and

i) coupling one or more targeting agents to a polymeric support and subsequently reacting the targeting agent-polymer intermediate with one or more molecules of the active substance to give a final complex containing one or more molecules of the active substance.

The above method may also include modification of the polymeric support to introduce functional groups capable of reacting either directly with the active substance or with a chemically-modified form of the active substance. The resulting polymer-active substance intermediate contains one or more molecules of the active substance, said intermediate being suitable for coupling to the targeting agent to give a complex capable of amplified delivery of the active substances.

Suitable extended spacers for the conjugation of the pharmaceutical, vitamin B_{12} or folate to the polymer matrix include : disuccinimidyl suberate (DSS), *bis*(sulfosuccinimidyl) suberate (BSS), ethylene glycol*bis*(succinimidylsuccinate) (EGS), ethylene glycol*bis*(sulfosuccinimidylsuccinate) (Sulfo-EGS), p-amino-phenylacetic acid, dithio*bis*(succinimidylpropionate) (DSP), 3,3'-dithio*bis*(sulfosuccinimidylpropionate) (DTSSP), disuccinimidyl tartarate (DST), disulfosuccinimidyl tartarate (Sulfo-DST), *bis*[2-(succinimidyloxycarbonyloxy)-ethylene]sulfone (BSOCOES), *bis*[2-(sulfosuccinimidooxycarbonyloxy)-ethylene]sulfone (Sulfo-BSOCOES), dimethyl adipimidate.2 HCl (DMA), dimethyl pimelimidate.2 HCl (DMP), dimethyl suberimidate.2 HCl (DMS), *N*-succinimidyl(4-iodoacetyl)aminobenzoate (SIAB) andsuccinimidyl 4-(p-maleimidophyl)butyrate (SMPB).

Suitable cross-linking agents for use in the preparation of thiol-cleavable biodegradable linkers include N-succinimidyl 3-(2-pyridyldithio)propionate (SPDP), iminothiolane, sulfosuccinimidyl 6-[3-(2-pyridyldithio) propionamido] hexanoate (Sulfo-LC-SPDP), succinimidyl 6-[3-(2-pyridyldithio) propionamido] hexanoate (LC-SPDP), sulfosuccinimidyl 6-[α-methyl-α-(2-pyridyldithio) toluamido]hexanoate (Sulfo-LC-SMPT), 1,4-di[3'-(2'-pyridyldithio)propionamido]butane (DPDPB), 4-succinimidyloxycarbonyl-α-methyl-α-(2-pyridyldithio)-toluene (SMPT) and dimethyl 3.3'dithiobispropionimidate.2 HCl (DTBP).

Suitable analogues of VB_{12} for derivatization prior to conjugation to the polymer include any variant or derivative of VB_{12} (cyanocobalamin) which possesses binding activity to

intrinsic factor. Preferred analogues of VB_{12} also include aquocobalamin, adenosylcobalamin, methylcobalamin, hydroxycobalamin, cyanocobalamin, carbanalide, and 5-methoxybenzylcyanocobalamin [(5-MeO)CN-Cbl] as well as the desdimethyl, monoethylamide and the methylamide analogues of all of the above. Other analogues include all alkyl cobalamins in which the alkyl chain is linked to the corrin nucleus by a direct CoC covalent bond. Other analogues include chlorocobalamin, sulfitocobalamin, nitrocobalamin, thiocyanatocobalamin, benzimidazolecyanocobalamin derivatives such as 5,6-dichlorobenzimidazole, 5-hydroxybenzimidazole, trimethylbenzimidazole, as well as adenosylcyanocobalamin [(Ade)CN-Cbl], cobalamin lactone, cobalamin lactam and the anilide, ethylamide, monocarboxylic and dicarboxylic acid derivatives of VB_{12} or its analogues.

Preferred derivatives of VB_{12} also include the mono-, di- and tricarboxylic acid derivatives or the propionamide derivatives of VB_{12}. Targeting molecules may also include analogues of VB_{12} in which the cobalt is replaced by zinc or nickel. The corrin ring of VB_{12} or its analogues may also be substituted with any substituent which does not effect its binding to IF, and such derivatives of VB_{12} or its analogues are part of this invention. Other derivatives of VB_{12} or its analogues which have a functional group which is able to react with the spacer compound are also part of the invention. Other derivatives and analogues of VB_{12} are discussed in Schneider, Z. and Stroinski, A., *Comprehensive VB12*; (Walter De Gruyter; Berlin, NY; 1987), the disclosure of which is incorporated herein by reference.

Still other derivatives of VB_{12} include those in which the 5'-hydroxyl group of the ribose moiety of the nucleotide ligand is modified. These derivatives include, but are not limited to derivatives formed by reaction with succinic anhydride, glutaric anhydride, *p*-maleimidophenyl isocyanate, oxirane, benzoquinone or cyanuric chloride. Alternatively derivatives can be formed by activation with 1,1'-carbonyldiimidazole and subsequent reaction with diamino-spacers, amino-acid-spacers, or alternatively with amino-alkyl chains to form hydrophobic derivatives (see Vitamin B_{12} derivatives and methods for their

production. PCT/AU99/00462, filed 11 June 1999, Russell-Jones, G. J., and McEwen, J. F.).

Reference to the term "folate" as used herein is to be considered in its broadest context and refers to the carboxylic acid anion of folic acid and, where not stated, the counter cation may be any suitable cation inlcuding pharmaceutically acceptable cations and may also include a proton, i.e. folic acid. The term "folate" may be taken to include reference to analogues of the folate molecule, such as methotrexate, and preferably where the analogue possesses some binding activity for the folic acid receptor.

Reference herein to "treatment" and prophylaxis" is to be considered in its broadest context. The term "treatment" does not necessarily imply that a host is treated until total recovery. Similarly, "prophylaxis" does not necessarily mean that the subject will not eventually contract a disease condition. Accordingly, treatment and prophylaxis include amelioration of the symptoms of a particular condition or preventing or otherwise reducing the risk of developing a particular condition. The term "prophylaxis" may be considered as reducing the severity of onset of a particular condition. "Treatment" may also reduce the severity of an existing condition.

The subject of the treatment or prophylaxis is a vertebrate host, preferably a veterinary , domestic or agricultural animal or human, or more preferably a mammal such as but not limited to human, primate, livestock animal (eg. sheep, cow, horse, donkey, pig) companion animal (eg. dog, cat) laboratory test animal (eg. mouse, rabbit, rat, guinea pig, hamster) captive wild animal (eg. fox, deer). Preferably the mammal is a human or primate. Most preferably the mammal is a human.

It will be understood that those skiled in the art will be able to employ method commonly known in the art for preparing suitable medicaments in concentrations and presented in forms appropriate to the administration of the folate complexes of the invention, optionally with other active agents as required, in suitable treatment regimes to achieve the desired physiological effect on the vertebrate host to be treated.

In accordance with these methods, the agents herein defined may be coadministered with one or more other compounds or molecules. For example, the pharmaceutical compositions of the invention may be administered in combination with other chemotherapeutic agents or other ameliorative acitve substances. By "administered in combination" is meant simultaneous administaration in the same formulation or in two different formulatinos via the same or different routs or sequential administration by the same or different routes. By "sequential" administratino is meant a time difference of from seconds, minutes, hours or days between the administration of the formulations. These agents may be administered in any order.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions (where water soluble) and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as licithin, by the maintenance of the required particle size in the case of dispersion and by the use of superfactants. The preventions of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimersal and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminium monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilisation. Generally, dispersions are prepared by incorporating the various sterilised active ingredient into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze-drying technique which yield a powder of the active ingredient plus any additional desired ingredient from previously sterile-filtered solution thereof.

When the active ingredients are suitably protected they may be orally administered, for example, with an inert diluent or with an assimilable edible carrier, or it may be enclosed in hard or soft shell gelatin capsule, or it may be compressed into tablets, or it may be incorporated directly with the food of the diet. For oral therapeutic administration, the active compound may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, gels, pastes, viscous colloidal dispersions, syrups, wafers, and the like. Such compositions and preparations should contain at least 1% by weight of active compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 5 to about 80% of the weight of the unit. The amount of active compound in such therapeutically useful compositions in such that a suitable dosage will be obtained. Preferred compositions or preparations according to the present invention are prepared so that an oral dosage unit form contains between about 0.1 μg and 2000 mg of active compound. In the case of compositions containing supplementary active ingredients, the dosages are determined by reference to the usual dose and manner of administration of the said ingredients.

The tablets, troches, pills, capsules and the like may also contain the following: A binder such as gum tragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; buffering agents such as sodium bicarbonate

to neutralise or buffer stomach acid; and a sweetening agent such a sucrose, lactose or saccharin may be added or a flavouring agent such as peppermint, oil of wintergreen, or cherry flavouring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar or both. A syrup or elixir may contain the active compound, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavouring such as cherry or orange flavour. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compound may be incorporated into sustained-release preparations and formulations.

Pharmaceutically acceptable carriers and/or diluents include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, use thereof in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the novel dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active material and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active material for the treatment of disease in living subjects having a diseased condition in which bodily health is impaired.

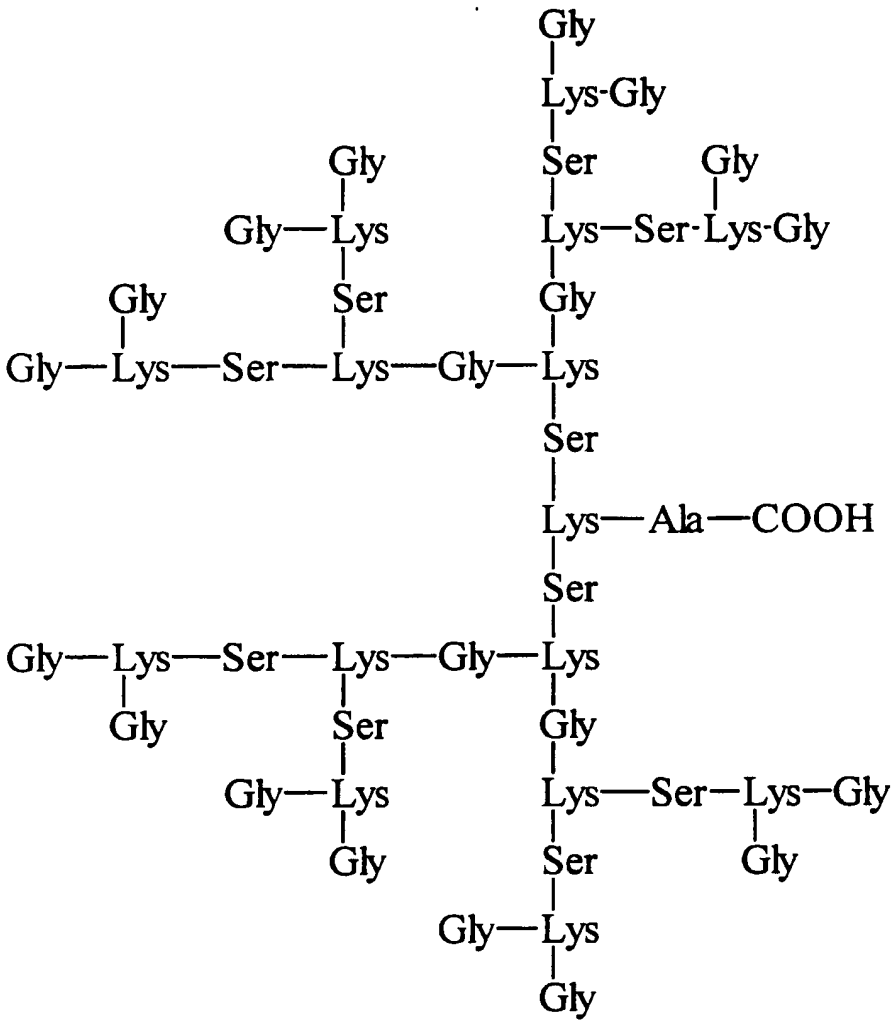
Administration of the agent in the form of a pharmaceutical composition may be performed by any convenient means. The agent of the pharmaceutical composition is contemplated to exhibit therapeutic activity when administered in an amount which depends on the particular case. Variation depends for example, on the human or animal and the agent chosen. Dosage regimes may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily, weekly, monthly or other suitable time intervals or the dose may be proportionally reduced as indicated by the exigencies of the situation. The agent may be administered in any suitable manner. Routes of administration include, but are not limited to, respiratorally, intratracheally, nasopharyngeally, intravenously, intraperitoneally, subcutaneously, intracranially, intradermally, intramuscularly, intraoccularly, intrathecally, intracereberally, intranasally, infusion, orally, rectally, *via* IV drip, patch and implant. With respect to intravenous routes, particularly suitable routes are via injection into vessels which supply the tumour or diseased organs. Peptides may also be installed into cavities for example the pleural or peritoneal cavity or injected directly into tumour tissues.

The present invention is further described with reference to the following examples which are in no way limiting on the scope of the invention.

Example 1. Synthesis of Multi-Lysine polymer 1 (MLP1)

A multi-lysine polymer (MLP1) of the formula $[(NH_2\text{-Gly})_4\text{-Lys}_2\text{-Ser}_2\text{-Lys}]_5$-Ala-COOH, was synthesized on an Applied Biosystems peptide synthesiser. More precisely this represents $[(NH_2\text{-Gly})_4\text{-Lys}_2\text{-Ser}_2\text{-Lys}]_4[\text{Gly}_4\text{-Lys}_2\text{-Ser}_2\text{-Lys}]$-Ala-COOH

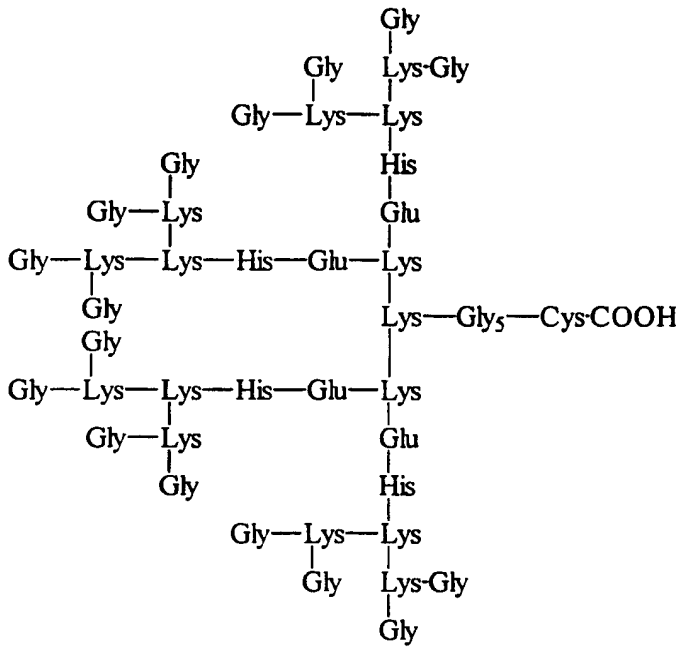
The formula $[(NH_2\text{-Gly})_4\text{-Lys}_2\text{-Ser}_2\text{-Lys}]_4[\text{Gly}_4\text{-Lys}_2\text{-Ser}_2\text{-Lys}]$-Ala-COOH can be represented as follows :



which shows the structure more precisely.

Example 2. Synthesis of Multi-Lysine polymer 2 (MLP2)

A multi-Lysine polymer (MLP2) of the general formula $[(NH_2\text{-Gly})_{16}\text{-Lys}_8\text{-Lys}_4\text{-His}_4\text{-Glu}_4\text{-Lys}_2\text{-Lys}]\text{-Gly}_5\text{-Cys-COOH}$ was synthesized on an Applied Biosystems peptide synthesiser. More precisely the structure can be represented as follows:



Example 3. Preparation of NHS-folate.

Folic acid (5g) was dissolved in 100 ml dry DMSO, plus 2.5 ml triethylamine. *N*-hydroxysuccinimide (2.6 gm) was added as a powder to the folic acid and reacted overnight with 4.7 gm dicyclohexylcarbodiimide at room temperature. The dicyclohexylurea was removed by filtration. The DMSO was concentrated under reduced pressure and heating, and NHS-folate precipitated with diethylether. The product, was washed several times with anhydrous ether, dried under vacuum and stored as a yellow powder.

Example 4. Formation of MLP-toxin conjugates using biodegradable cross-linkers.

There are many toxins which could be used for formation of folate-MLP-toxin conjugates, including momordin, psuedomonas exotoxin A, ricin and abrin.

A general method for the formation of folate-MLP-toxin conjugates is described below. Conjugates are prepared in which the covalent linker contains a biodegradable disulfide bond, which would be reduced *in vivo*, presumably by intracellular glutathione in the tumor cell, thereby releasing the active substance after transport from the serum into the tumor cell. Briefly, MLP1 or MLP2 was reacted with N-succinimidyl 3-(2-pyridyldithio)propionate (SPDP). The dithiopyridyl-MLP (DTP-MLP) product was purified by RP-HPLC. A free thiol was introduced onto the toxin by a two step procedure in which the toxin was firstly reacted with SPDP, after which the thiopyridyl group was with mercapto-ethanol. The product was purified by RP-HPLC. Alternatively free thiol was introduced into the toxin directly by reaction with iminothiolane. The thiolated product (SH-HN^+toxin) was purified by RP-HPLC. Formation of the disulfide linked MLP-toxin conjugates was achieved by reaction of the thiolated toxin derivative with DTP-MLP in 2.5% acetic acid for 24 hours. The conjugated material was purified by Sephadex G-25 chromatography, followed by RP-HPLC.

Folate was linked to the polymer-toxin complexes by reacting adipyl-hydraxidylfolate derivative with the complex using EDAC. The reacted product was purified by RP-HPLC.

Example 5. Synthesis of Chlorambucil-tetra-peptide prodrugs.

A chlorambucil-tetra peptide prodrug was synthesized that contained a sequence cleavable by intracellular lysosomal enzymes. The tetra peptide, Gly-Phe-Leu-Gly was synthesized by standard Merrifield synthesis. Chlorambucil (61 mg) and carbonyl diimidazole (32 mg) were dissolved in dry DMF (300 µl) and the solution stirred at room temperature for 1 hour. A solution of Gly-Phe-Leu-Gly (75 mg, in 300 µl DMF) was added dropwise to the active ester, followed by diisopropylethylamide (DIEA) (150 µl), and the solution was allowed to react overnight. The unreacted chlorambucil was extracted in the DCM phase of a water/DCM wash, and the Chlorambucil-Gly-Phe-Leu-Gly product was isolated from the aqueous phase by chromatography on RP-HPLC using a linear gradient of 5-100% acetonitrile.

Example 6. Synthesis of Chlorambucil-Gly-Gly prodrugs.

A chlorambucil-tetra peptide prodrug was synthesized that contained a sequence that is not cleavable by intracellular lysosomal enzymes. The dipeptide, Gly-Gly was purchased from Sigma. Chlorambucil (61 mg) and carbonyl diimidazole (32 mg) were dissolved in dry DMF (300 µl) and the solution stirred at room temperature for 1 hour. A solution of Gly-Gly (75 mg, in 300 µl DMF) was added dropwise to the active ester, followed by diisopropylethylamide (DIEA) (150 µl), and the solution was allowed to react overnight. The unreacted chlorambucil was extracted in the DCM phase of a water/DCM wash, and the Chlorambucil-Gly- Gly product was isolated from the aqueous phase by chromatography on RP-HPLC using a linear gradient of 5-100% acetonitrile.

Example 7. Synthesis of cobalamin-carboxypeptidase conjugate.

Carboxypeptidase A (Boeringher) (10 mg) was precipitated from solution by centrifugation. The resultant precipitate was resuspended in distilled water and dialysed extensively overnight against DW. The "e"monocarboxylic acid isomer of vitamin B_{12} was dissolved at 100 mg/ml in DMF (5 mg in 50 µl). DIEA (1.5 µl) and TSTU (O-(N-succinimidyl)-N,N,N',N'-tetramethyluronium tetrafluoroborate) (1.8 mg in 10 µl DMF) was added and allowed to stir for 10 minutes. The activated ester was then added to the carboxypeptidase A in 2% $NaHCO_3$ and allowed to react overnight. Unreacted vitamin B_{12} was removed by extensive dialysis against distilled water.

Industrial Applications

The present invention provides a simple and novel technique for the specific dual targeting of diseased tissue and cells and the amplification of drug/pharmaceutical delivery to the tissues and cells. The present invention also provides novel compositions for use in targeting those tissues and cells.

The claims defining the invention are as follows:

1. A pharmaceutical composition comprising a first vitamin complex and a second vitamin complex, wherein the vitamin of the first complex is different to the vitamin of the second complex and wherein both vitamins are complexed to the same agent or active substance.

2. A pharmaceutical composition of claim 1, wherein the vitamins are selected from the group consisting of folic acid, vitamin B_{12}, riboflavin, biotin and derivatives and analogues thereof.

3. A pharmaceutical composition of claim 1, wherein the vitamins are folic acid and vitamin B_{12} or derivatives thereof.

4. A pharmaceutical composition of claim 1, wherein the agent or active substance is selected from the group consisting of hormones, drugs, prodrugs, enzymes, proteins, peptides, toxins, immunogens and DNA or RNA analogues.

5. A pharmaceutical composition of claim 1, wherein the agent or active substance is encapsulated in a nanoparticle or microsphere vitamin complex or linked to a vitamin-polymer complex.

6. A pharmaceutical composition comprising a first vitamin complex and a second vitamin complex, wherein the vitamin of the first complex is different to the vitamin of the second complex and wherein both vitamins are each complexed to different agents or active substances.

7. A pharmaceutical composition of claim 6, wherein the vitamins are selected from the group consisting of folic acid, vitamin B_{12}, riboflavin, biotin and derivatives and analogues thereof.

8. A pharmaceutical composition of claim 6, wherein the vitamins are folic acid and vitamin B_{12} or derivatives thereof.

9. A pharmaceutical composition of claim 6, wherein the agent or active substance is selected from the group consisting of hormones, drugs, prodrugs, enzymes, proteins, peptides, toxins, immunogens and DNA or RNA analogues.

10. A pharmaceutical composition of claim 6, wherein the agent or active substance is encapsulated in a nanoparticle or microsphere vitamin complex or linked to a vitamin-polymer complex.

11. A pharmaceutical composition of claim 6, wherein the second vitamin complex is a prodrug of a cytotoxic agent and the first vitamin complex is an enzyme or reagent adapted to transform the prodrug into the cytotoxic agent or to release the cytotoxic agent.

12. A pharmaceutical composition of claim 11, wherein the first vitamin complex and the second vitamin complex comprise any one of the following vitamin conjugate pairs:

a) Cbl-enzyme + Folate-substrate-cytotoxin,
b) Folate-enzyme + Cbl-substrate-cytotoxin,
c) Cbl-enzyme + Folate-Polymer-substrate-cytotoxin,
d) Folate-enzyme + Cbl-Polymer-substrate-cytotoxin,
e) Cbl-enzyme + Folate-NP-substrate-cytotoxin,
f) Folate-enzyme + Cbl-NP-substrate-cytotoxin, and
g) Cbl-NP-Enzyme + Folate-NP-substrate-cytotoxin.

13. A method for the manufacture of a pharmaceutical composition of claim 1 or claim 6 which method comprises admixing the first vitamin complex, the second vitamin complex and optionally one or more further vitamin complexes together with a pharmaceutically acceptable carrier, excipient, diluent and/or adjuvant.

14. A pharmaceutical composition prepared by a method of claim 13.

15. A method for the treatment, prophylaxis or amelioration of disease, preferably cancer, which comprises administering to a subject a therapeutically effective amount of a first vitamin complex and a second vitamin complex wherein the vitamin of the first complex is different to the vitamin of the second complex and wherein both vitamins are complexed to the same agent or active substance.

16. A method for the treatment, prophylaxis or amelioration of disease, preferably cancer, which comprises administering to a subject a therapeutically effective amount of a first vitamin complex and a second vitamin complex wherein the vitamin of the first complex is different to the vitamin of the second complex and wherein both vitamins are each complexed to different agents or active substances.

17. A method of claim 15 or claim 16, wherein the subject is a vertebrate host selected from the group consisting of veterinary, domestic and agricultural animals and humans.

18. A method of claim 15 or claim 16, wherein the administration of the first vitamin complex and the second vitamin complex is either simultaneous or sequential.

19. Use of a first vitamin complex and a second vitamin complex wherein the vitamin of the first complex is different to the vitamin of the second complex and wherein both vitamins are complexed to the same agent or active substance in the preparation of a medicament for the treatment, prophylaxis or amelioration of disease, preferably cancer.

20. Use of a first vitamin complex and a second vitamin complex wherein the vitamin of the first complex is different to the vitamin of the second complex and wherein both vitamins are each complexed to different agents or active substances in the manufacture of a medicament for the treatment, prophylaxis or amelioration of disease, preferably cancer.

21. A kit for the delivery of one or more agents or active substances to a subject, said kit comprising a first vitamin complex and a second vitamin complex wherein the vitamin of the first complex is different to the vitamin of the second complex and wherein both vitamins are complexed to the same agent or active substance.

22. A kit for the delivery of agents or active substances to a subject, said kit comprising a first vitamin complex and a second vitamin complex wherein the vitamin of the first complex is different to the vitamin of the second complex and wherein both vitamins are each complexed to different agents or active substances.

23. A kit of claim 22, wherein the vitamins are selected from the group consisting of folic acid, vitamin B_{12}, riboflavin, biotin and derivatives and analogues thereof.

24. A kit of claim 22, wherein the vitamins are folic acid and vitamin B_{12} or derivatives thereof.

25. A kit of claim 22, wherein the agent or active substance is selected from the group consisting of hormones, drugs, prodrugs, enzymes, proteins, peptides, toxins, immunogens and DNA or RNA analogues.

26. A kit of claim 22, wherein the agent or active substance is encapsulated in a nanoparticle or microsphere vitamin complex or linked to a vitamin-polymer complex.

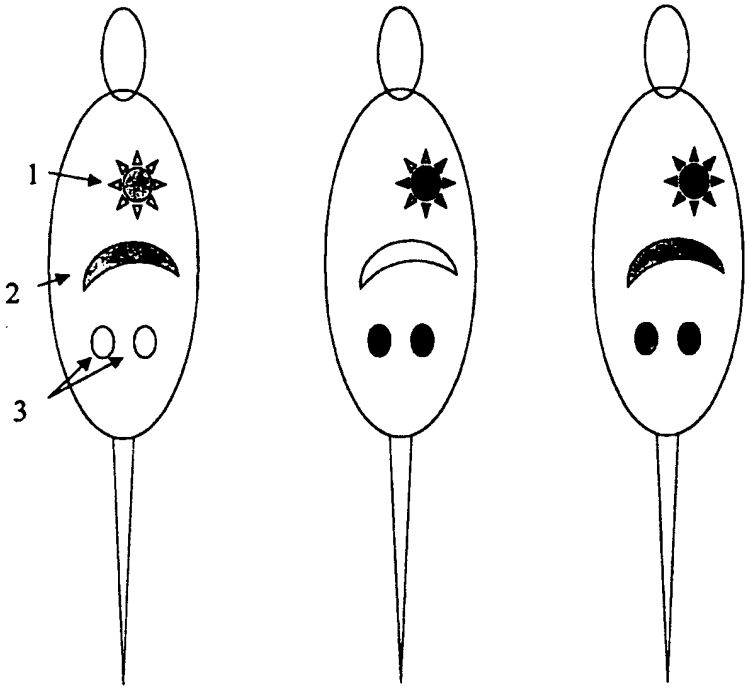
27. A kit of claim 21, wherein the cytotoxic agent is selected from the group consisting of alkylating agents including chlorambucil, cyclophosphamide, melphalan, cyclopropane; anthracycline antitumor antibiotics including doxorubicin, daunomycin, adriamycin, mitomycin C, 2-(hydroxymethyl)anthraquinone; antimetabolites including methotrexate, dichloromethatrexate, cisplatin, carboplatin; metallopeptides containing platimun, copper, vanadium, iron, cobalt, gold, cadmium, zinc and nickel and other agents including DON, thymidine, pentamethylmelamin, dianhydrogalactitol, 5-Methyl-THF, anguidine,

maytansine, neocarzinostatin, chlorozotocin, AZQ, 2'deoxycoformycin, PALA, AD-32, *m*-AMSA and misonidazole.

28. A kit of claim 21, wherein the second vitamin complex incorporates a prodrug of a cytotoxic agent and the first vitamin complex incorporates an enzyme or reagent adapted to transform the prodrug into the cytotoxic agent or to release the cytotoxic agent.

29. A kit of claim 28, wherein the first vitamin complex and the second vitamin complex comprise any one of the following vitamin conjugate pairs:

a) Cbl-enzyme + Folate-substrate-cytotoxin,
b) Folate-enzyme + Cbl-substrate-cytotoxin,
c) Cbl-enzyme + Folate-Polymer-substrate-cytotoxin,
d) Folate-enzyme + Cbl-Polymer-substrate-cytotoxin,
e) Cbl-enzyme + Folate-NP-substrate-cytotoxin,
f) Folate-enzyme + Cbl-NP-substrate-cytotoxin, and
g) Cbl-NP-Enzyme + Folate-NP-substrate-cytotoxin.



1A 1B 1C

Figure 1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU00/00618

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. [7]: A61K 47/48, A61P 35/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC: A61K 47/48

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
AU: IPC AS ABOVE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WPAT: VITAMIN, FOLIC, RIBOFLAVIN, VITAMIN B, A61K 47/48
CAPLUS: FOLIC ACID, RIBOFLAVIN, VITAMIN B, VITAMIN B12, BIOTIN, COBALAMIN, MLP, COMPLEX, POLYMER, TUMOR, MULTILYSINE, POLYLYSINE, CARBOXYPEPTIDASE DIPEPTIDE, TETRAPEPTIDE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 99/65930 (BIOTECH AUSTRALIA PTY LTD) 23 December 1999, whole Document	1-27
A	WO 94/27613 (BIOTECH AUSTRALIA PTY LTD) 8 December 1994, whole document	1-27
A	WO 94/27641 (BIOTECH AUSTRALIA PTY LTD) 8 December 1994, whole document	1-27

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 July 2000	31 JUL 2000
Name and mailing address of the ISA/AU	Authorized officer
AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	S. CHANDRA Telephone No : (02) 6283 2264

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU00/00618

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 97/14740 (RECEPTAGEN CORPORATION et al) 24 April 1997, whole document	1-27
A	WO 95/27723 (MORGAN, A et al) 19 October 1995, whole document	1-27
A	WO 99/65529 (ALZA CORPORATION) 23 December 1999, whole document	1-27
A	US 4774089 (ASHMEAD) 27 September 1988, whole document	1-27
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INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/AU00/00618

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

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END OF ANNEX